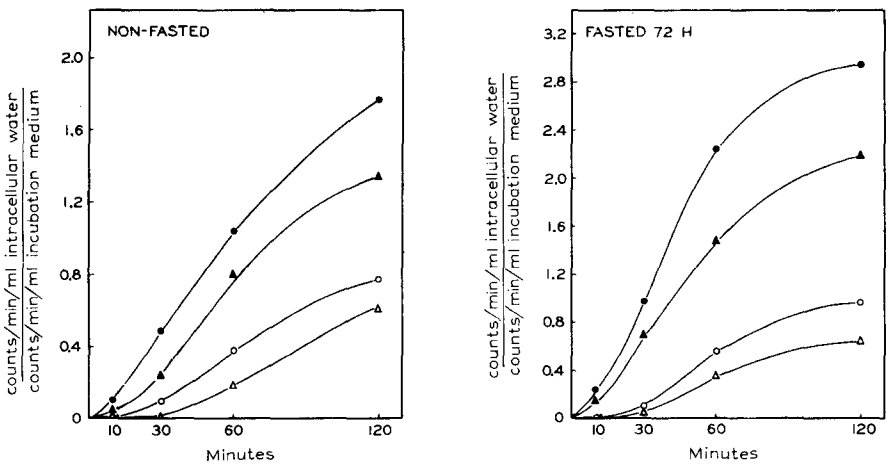
Stimulation of amino acid transport by insulin in the isolated rat diaphragm*

Insulin has been found to stimulate the incorporation of glycine, L-phenylalanine[1] and L-alanine[2] into proteins of the isolated rat diaphragm. It is not known whether this response is secondary to an accelerated rate of amino acid transport or to a direct stimulation of protein synthesis. In order to test the former possibility, a non-utilizable amino acid analogue[3], α-aminoisobutyric acid (AIB), has been selected as a model with which to study amino acid penetration into skeletal muscle.

AIB labelled with ^{14}C in the carboxyl group, with a specific activity of $4.2 \cdot 10^8$ counts/min/mmole, was synthesized by the method of NOALL AND CHRISTENSEN[4]. The rate of intracellular penetration was determined by incubating an "intact" diaphragm preparation, the characteristics of which have been described by KIPNIS AND CORI[5], for various time periods in volumes of buffer sufficiently large to permit the external concentration of amino acid to remain constant. Control experiments showed that AIB was not incorporated into the protein or lipid fraction of rat diaphragm or oxidized to CO_2. From 95 to 100% of the radioactivity originally added was recovered after several hours of incubation. Total tissue radioactivity can be extracted by grinding the diaphragm in 5% trichloroacetic acid or 0.008 M acetic acid. Chromatograms of these extracts showed that all of the radioactivity was contained in one spot which coincided with authentic AIB. Penetration was measured in diaphragms obtained from fed and fasted animals in the presence and absence of glucose and insulin.

The results are shown in Figs. 1 and 2 where the ratio of counts/min/ml intracellular water to counts/min/ml incubation medium is plotted against time of incubation. Insulin produces a three- to five-fold increase of the rate of amino acid penetration in both the fasted and non-fasted preparation in the presence or absence of glucose. The dietary status also markedly influences the AIB penetration, being twice as rapid in the diaphragms of 72-h fasted rats than in those of non-fasted rats. Glucose, in a molar ratio to AIB of 300 to 1, resulted in a 30% decrease in the rate of penetration in the presence and absence of added insulin. Under the experimental conditions employed, AIB was concentrated intracellularly against a concentration gradient. Since the periods



Figs. 1 and 2. Diaphragms were incubated at 37° under continuous oxygenation in 30 ml Krebs-Henseleit phosphate buffer, pH 7.4. Pre-incubations in glucose-free buffer for 15 min prior to the experimental period were performed in order to permit utilization of the glucose remaining in the tissue following its excision. Concentration of the various substances used were: AIB, $3.2 \cdot 10^{-5}$ M; glucose, 0.01 M; insulin (Iletin-Lilly), 0.4 units/ml incubation medium. Aliquots of tissue extract, prepared by grinding the tissue in 10 vol. 0.008 M acetic acid, and of incubation medium, diluted with equal vol. 10% egg albumin, were plated, dried, and assayed with a gas-flow proportional counter. All results were corrected to infinite thinness. Counts/min/ml intracellular water were calculated by subtracting the radioactivity corresponding to the extracellular volume from the total tissue-water content. ● insulin added, ▲ glucose and insulin added, △ glucose added, ○ neither insulin nor glucose added.

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of incubation were not long enough for the attainment of a constant ratio of concentrations (intracellular/extracellular), the effect of insulin and fasting on the final equilibrium could not be determined.

These results indicate that future concepts of insulin action must be broadened to include the stimulation of both sugar and amino acid penetration. Further studies of amino acid penetration and intracellular-concentrating processes and the influence of dietary and hormonal factors thereon are currently in progress.

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Cytochrome reactions in *Chromatium*

Spectrophotometric investigations[1,2] of *Rhodospirillum rubrum* have indicated that cytochromes are involved in electron transport during bacterial photosynthesis. The present study of the purple sulfur bacterium *Chromatium*, strain *D*, substantiates this concept.

The bacteria were grown at 29° under anaerobic conditions in a liquid inorganic medium containing sulfide, thiosulfate, and bicarbonate as substrates. Tungsten lamps furnished illumination. The bacterial cultures were examined after most of the sulfur had disappeared from the cells. A double-beam spectrophotometer[3] was used to record absorption changes upon irradiation of a sample with near infrared ($\lambda > 700$ mμ) light; a split-beam spectrophotometer[4] was used to obtain the difference spectra of pairs of samples in the dark.

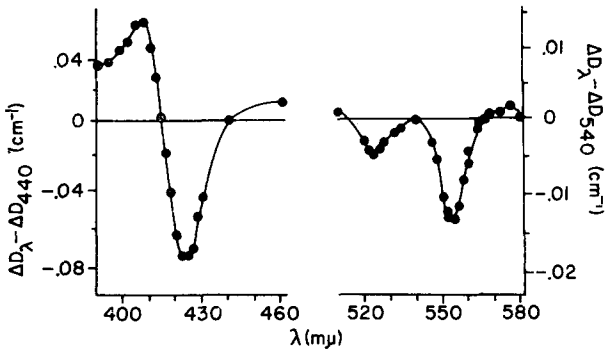

Fig. 1. Absorption-spectrum changes upon irradiation under anaerobic conditions. The trough locations are 423, 524, and 553 mμ. The ratio $(\Delta D_{423} - \Delta D_{440})/(\Delta D_{553} - \Delta D_{540})$ is approximately 4.5. Two different cultures were used for the two spectral intervals shown.

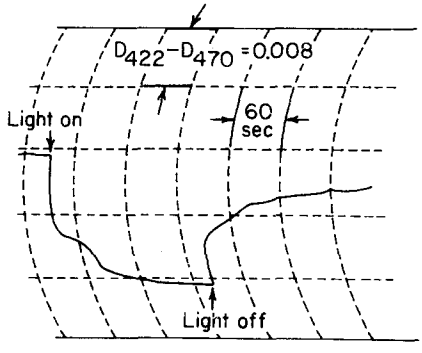

Fig. 2. Kinetics of the anaerobic light effect. The change in absorption at 422 mμ *minus* the change at 470 mμ has been recorded.

When anaerobic suspensions were irradiated with near infrared, the change in absorption spectrum for the region 390 to 580 mμ indicated the oxidation of cytochrome (Fig. 1). The kinetics of the light-on transition were diphasic, and the kinetics of the light-off transition were triphasic (Fig. 2). The differences between the spectra of these various phases showed that more than one cytochrome species was involved in the total light reaction.